Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Merritt WM, Lin YG, Han LY, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med 2008;359:2641-50.

Supplementary information

Methods

Authors Contribution List

1. Study design
2. Data gathering
3. Data analysis
4. Vouches for data
5. Wrote manuscript
6. Decision to publish
7. Prepared first draft of manuscript

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Cell line source. The HeyA8 and SKOV3ip1 cell lines were obtained from Dr. Isaiah J. Fidler (M.D. Cancer Center, Houston, TX); A2780-PAR cell line was obtained from Dr. Thomas Hamilton at the Fox Chase Cancer Center (Bethesda, MD); EG and IGROV cell lines were obtained from Dr. Richard Buller (University

of Iowa Hospitals, Iowa City, IA); and OVCAR3 cell line was obtained from the American Type Cell Collection (Ref. num. HTB-161). The 222 cell line was obtained from Dr. Benjamin Bonavida (UCLA School of Medicine, Los Angeles, CA). The OVCAR-420 cell line was obtained from Dr. Sam Mok (Brigham and Women's Hospital, Boston, MA). The nontransformed ovarian surface epithelial cell line HIO-180 was given by Dr. Andrew Godwin at the Fox Chase Cancer Center (Philadelphia, PA).

Western blot analysis. After protein loading, bands were separated by SDS-PAGE and transferred to nitrocellulose paper. Immunoblotting was performed as previously described[36] using either mouse anti-Dicer (1:1000; Abcam, Cambridge, MA), mouse anti-Drosha (1:500; Abcam), or rabbit anti-Galectin-3 antibodies (provided by Dr. Avraham Raz, Karmanos Cancer Center, Wayne State University, Detroit, MI).

Results

Dicer and Drosha expression in ovarian cancer cell lines. Compared to the non-transformed ovarian surface epithelial cells, HIO-180, Dicer and Drosha mRNA expression was increased by 2.01 to 3.41 fold and 1.08 to 1.87 fold, respectively, in half of the ovarian cancer cell lines (Supplementary Figure 2A). In the other 4 cell lines, mRNA expression of Dicer (2.0 to 12.5 fold) and Drosha (1.1 to 15.3 fold) was decreased. Protein expression was decreased in 5 of 8 cell lines (1.20

to 5.33 fold) for Dicer, and in 3 of 8, for Drosha (1.09 to 2.23 fold; Supplementary Figures 2B and C).

Mutational analysis of Dicer and Drosha in ovarian cancer cell lines. Genomic DNA from cell lines with low (HeyA8 and SKOV3ip1) and high (OVCAR3 and A2780-PAR) Dicer and Drosha expression levels was analyzed for mutations. Re-sequencing of the coding exons demonstrated two synonymous single-nucleotide polymorphisms for both Dicer and Drosha in all four cell lines. Two different non-synonymous mutations were noted in Drosha in OVCAR3 and A2780-PAR cell lines. A splice-site mutation was discovered for Drosha in the SKOV3ip1 cell line. RT-PCR failed to demonstrate truncation of Drosha in any of the four cell lines examined by mutational analysis (data not shown).

Supplementary Table 1. Distribution of Dicer and Drosha mRNA levels in ovarian cancer tumors

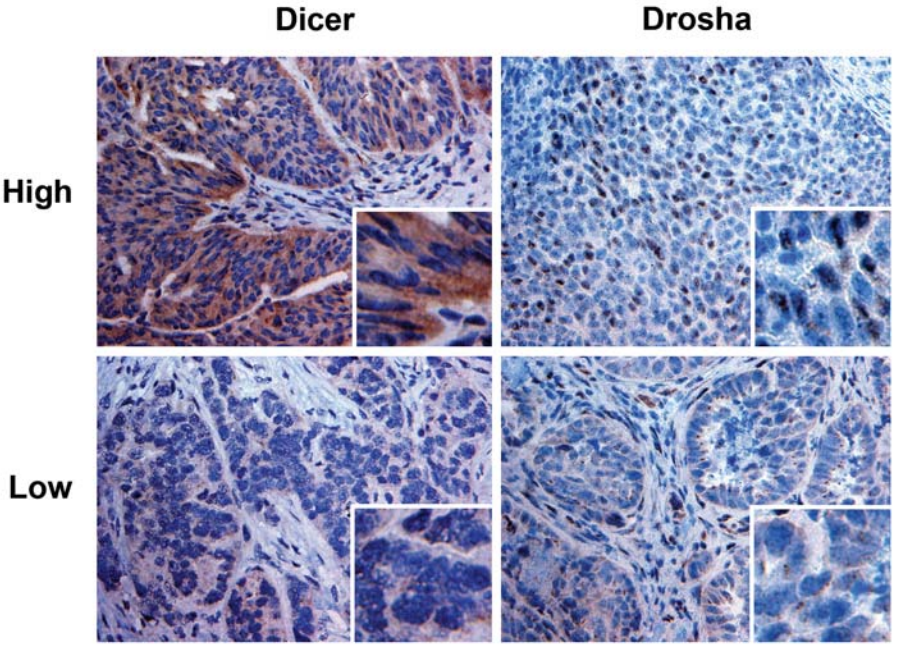
		N (%) N=111
Individual	**Low Dicer**	**66 (60)**
	Low Drosha	**57 (51)**
Joint	**Low Dicer and Drosha**	**43 (39)**
	High Dicer, Low Drosha	**14 (12)**
	Low Dicer, High Drosha	**23 (21)**
	High Dicer and Drosha	**31 (28)**

Supplementary Figure 1. Immunohistochemical analysis of Dicer and Drosha expression in human tumors. Representative images (200x original magnification) of tumors with high or low Dicer and Drosha immunhistochemical staining (magnification of staining represented in inset).

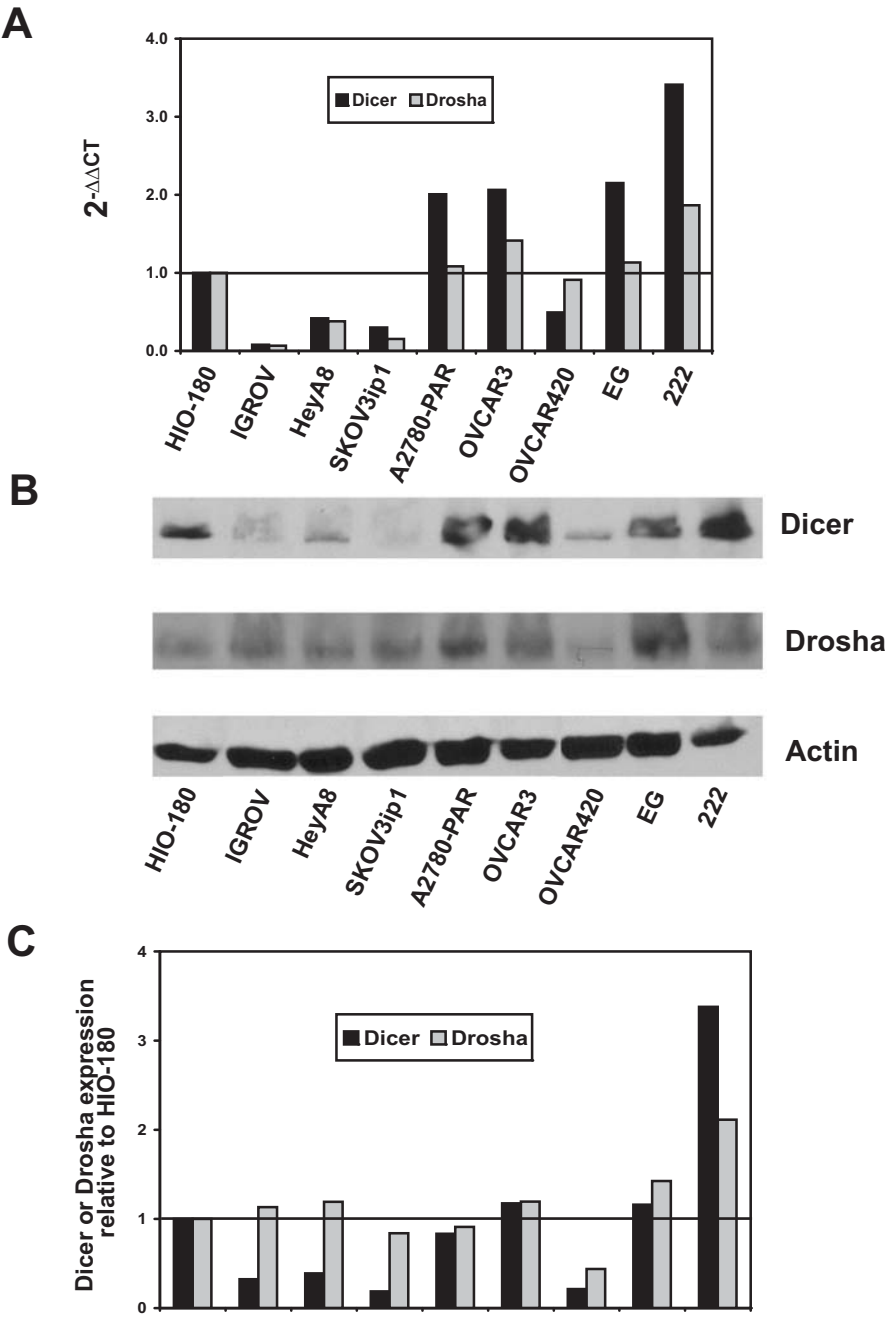
Supplementary Figure 2. Dicer and Drosha expression in ovarian cell lines. **A**) Quantitative RT-PCR analysis of Dicer and Drosha mRNA expression in a non-transformed ovarian epithelial cell line (HIO-180) and invasive ovarian epithelial cancer cell lines. $2^{-\Delta\Delta CT}$= Ratio of Dicer and Drosha expression relative to that in the non-transformed HIO-180 cell line [16]. **B**) Western blot analysis of Dicer and Drosha. **C**) Densitometry analysis.

Supplementary Figure 3. Kaplan-Meier survival curve for patients with invasive epithelial ovarian cancer in relation to tumor expression of both Dicer and Drosha.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3